

Role of Liver Kinase B1 and its downstream kinases as regulators of estrogen receptor signaling in estrogen receptor-positive breast cancer

Bakhtawar Tufail¹, Laki Buluwela², Simak Ali², David H. Drewry³, Van H. Barnes^{1,4},
Elizabeth C. Martin^{1,4}, Matthew E. Burow^{1,4}

¹Department of Medicine, Section of Hematology and Oncology, Tulane University, New Orleans, LA, 70118, USA.

²Department of Surgery and Cancer, Imperial College London, UK.

³Structural Genomics Consortium, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, North Carolina 27599, USA.

⁴Tulane Cancer Center, Tulane University, New Orleans, LA 70112

Estrogen receptor positive (ER+) breast cancer accounts for ~70% of breast cancers, relying on estrogen-ER binding for growth and progression. Although endocrine therapy is the mainstay treatment, de novo or acquired resistance in ~40% of patients, concomitant with loss of estrogen (E2) sensitivity, remains a challenge. ER interacts with kinase signaling pathways regulating cell survival, growth, and endocrine resistance. While pathways such as AKT-mTOR and MAPK are well studied, others remain underexplored, and therapy resistance remains an issue. Liver Kinase B1 (LKB1), a highly conserved tumor suppressor, functions as a co-activator of ER-dependent transcription; however, it doesn't directly phosphorylate ER. Paradoxically, LKB1 deletion in ER+ breast cancer cells promotes resistance to endocrine therapies, suggesting that the ER-LKB1 interaction may be mediated through downstream kinase signaling pathways. LKB1 phosphorylates 14 downstream adenosine monophosphate-related kinases (ARKs), regulating cellular transcription, metabolism, and growth. Although evidence suggests crosstalk between LKB1-ARKs and ER signaling pathways, a complete understanding of the LKB1-ARKs axis and ER regulation is lacking. It is hypothesized that *"LKB1 regulates ER-dependent transcription through downstream ARKs, influencing endocrine response. Further, identification of ARKs regulating ER signaling will provide novel candidate biomarkers and therapeutic targets in ER+ breast cancer"*. To investigate this, CRISPR-mediated LKB1 knockdown (KD) in ER+ MCF7 cells, followed by RNA sequencing, revealed broad transcriptional reprogramming, including altered ARKs expression, significantly reduced ER expression, and downregulation of hallmark estrogen-response pathways. To define the E2 response, LKB1-KD cells were evaluated under estrogen-deprived conditions with or without E2. Compared to the control, LKB1-KD cells showed significantly reduced ER and ER-dependent gene expression and substantial ARK rewiring, including increased NUA2 and MARK1 expression under both conditions. To determine a functional role of ARKs, ER+ cells were treated with ARK inhibitors and assessed for cell density. Results demonstrated that in estrogen-deprived media, SIK inhibition reduced cell density in MCF7 cells, an effect attenuated by E2. Together, these findings implicate a potential crosstalk between the LKB1-ARK axis and ER signaling through AMPK-related kinases.